

The Action of Anæsthetics on Living Tissues. Part II.—The Frog's Skin.

By N. H. ALCOCK, M.D.

(Communicated by A. D. Waller, M.D., F.R.S. Received April 9,—Read May 10, 1906.)

(From the Physiological Laboratories of the University of London and St. Mary's Hospital Medical School.)

CONTENTS.

- I. Introduction.
- II. Experiments—Series I.—Anæsthetics to inner and outer surfaces.
- III. Series II.— CHCl_3 to outer surfaces in parallel.
- IV. Series III.—Electrical resistance of skin after CHCl_3 .
- V. Deductions. Relations to Part I. Alternative theories.

Introduction.

The currents given by the frog's skin and their variations after excitation both directly and through the nerves have already been studied by many authors,* but, as far as I can determine, no one has up to the present time observed the action of anæsthetics on the resting current, and as these phenomena form a fitting sequel to those occurring in nerve (Part I of this series),† they form the subject of this paper. It is to be observed that from the nature of the object one of the possible alternative hypotheses in the case of nerve is here excluded, and it is therefore now legitimate to take a further step in explanation of the action of chloroform on the tissues generally.

Experiments.

Series I.

A chamber was constructed of vulcanite, through the bottom of which three non-polarisable electrodes projected. A movable partition of modelling wax, luted with moist china-clay, divided one electrode, A, from the

* Most of the references to the numerous papers on the frog's skin are to be found in the article by Waymouth Reid in Schäfer's 'Text-book of Physiology,' vol. 1, pp. 669—691. A criticism of the various theories is in the paper by Bayliss and Bradford, 'Journ. Physiol.,' 1886, vol. 7, p. 223. See also Waller, 'Signs of Life,' 1905.

† 'Roy. Soc. Proc.,' B, vol. 77, 1906, p. 267.

other two, C and B (fig. 1). The skin was placed with the outer surface resting on A and C, the movable upper portion of the partition adjusted, and contact effected with the electrode B through a bundle of linen threads soaked in $m/10$ NaCl solution. In this series the electrode C served merely as a support, and the potential differences between A and B were alone considered.

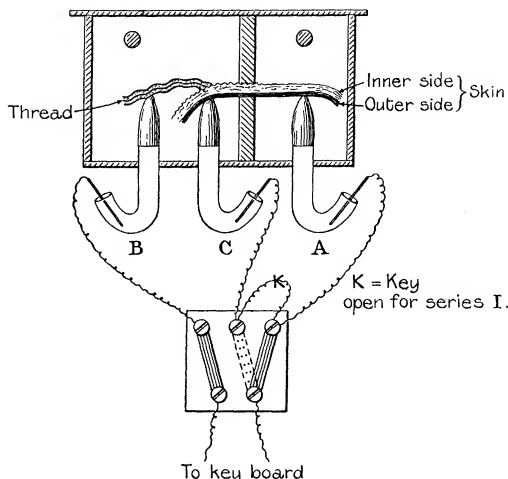


FIG. 1.—Chamber and Electrodes for Series I and II.

To make a preparation the frog is first killed by pithing, and the skin of the back cut into two longitudinal strips 3 to 5 mm. wide, one on either side of the middle line. These strips are very gently lifted up and the connections below severed, special care being taken to avoid stretching or other injury. A preparation of this kind gives a "resting" potential difference of from 0.0200 to 0.1100 volt,* sometimes exceeding this latter figure, the value varying according to the avoidance of injury and the condition of the frog, freshly caught frogs giving much higher values than those kept in captivity for some time. Any injury diminishes the potential indefinitely, and it is desirable to test the preparations from time to time by examining the skin *in situ*. If one electrode be placed very gently on the external surface of the skin and another on any portion of the subcutaneous tissue, one obtains the "resting" potential with the minimum of manipulation, and the value from the excised strip should not be much below this.†

* Du Bois-Reymond ('Untersuchungen,' vol. 2, Abth. 2, pp. 9—20) gives a maximum value of 0.0561 volt and a minimum of 0.0044; Bayliss and Bradford (*loc. cit.*), 0.25 and 0.03 volt.

† There are many points still remaining to be determined with regard to the resting current in relation to variations in the conditions of observation: some of these are under consideration.

In the Experiments 502 to 506 inclusive, moist air containing about 16 per cent. of CHCl_3 vapour delivered from the apparatus previously described (Part I)* was applied *first* to the part of the chamber containing electrode B, which touched the inner surface, and *secondly* to electrode A, which touched the outer surface, the electrode not receiving CHCl_3 being ventilated with moist air. The figures plotted out in fig. 2 are the successive readings of the potentiometer balancing the E.M.F. from the skin, as in the previous experiments on nerve.†

It will be seen that CHCl_3 to B produces a minimal effect,‡ while CHCl_3 to A causes an immediate and very rapid fall to the zero line. In Experiments 500 and 501 (fig. 3) the order was reversed, CHCl_3 to A produced a fall of potential exactly as in fig. 2, CHCl_3 to B having again but a very slight action. The conclusion is therefore—

- (i) CHCl_3 to the outer surface of the skin abolishes the current.
- (ii) CHCl_3 to the inner surface of the skin has no action (neglecting the minimal effect noted above). From these conclusions follows another—
- (iii) The apparatus giving the current is placed at or very near the outer surface.

Further inferences can be drawn ; they are considered later.

Series II.

In Experiments 507 and 508 advantage was taken of the third electrode C which touched the outer surface opposite the electrode B, and measurements were taken by means of the ABC key of the potential between A and B, B and C, and A + C (in parallel) and B. The intervals between successive observations of the same pair of electrodes were about one minute, and the readings were in the order given, so that the readings for BC and (A + C) B are in each case a little later than those of AB. In both experiments all three readings showed the gradually lessening increase characteristic of a normal preparation under the conditions of experiment. CHCl_3 was then applied to the chamber containing electrodes BC.

* *Loc. cit.*, p. 271.

† The figures have been corrected for electrode current ; the error of reading is about ± 0.0001 volt. The potentiometer error is rather more, but as this is constant for all the experiments, no correction has been applied.

‡ This is probably due in part to a slight escape of CHCl_3 vapour to A, in part also because, although the outer surface has a high resistance (*vide infra*), this is not infinite.

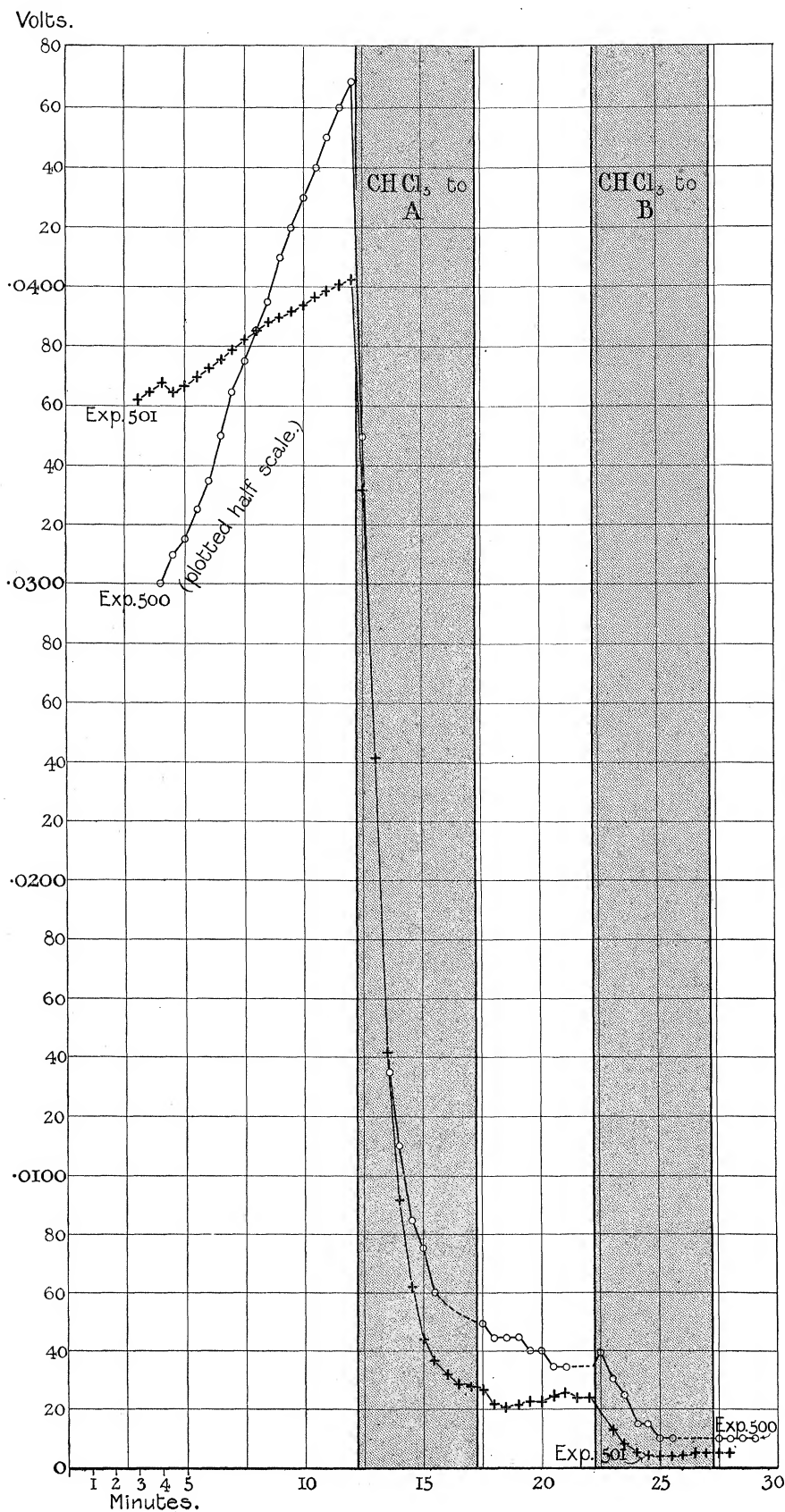


FIG. 3.—Series I. CHCl₃ (16 per cent.) to Parts of Skin. A = Outer Surface to Electrodes; B = Inner Surface. Experiment 501, CHCl₃ for 5-minute intervals; Experiment 500 for 3.5-minute intervals.

Experiment 507.

AB.	BC.	(A + C) B.
volt.	volt.	volt.
0·0299	0·0349	0·0338
355	350	354
370	361	369
382	354	366
390	357	369
396	358	372
CHCl ₃ to BC.		
410	+ 282	312
410	200	230
415	120	190
420	60	150
420	30	130
423	15	115
424	+ 5	110
426	— 2	102
CHCl ₃ off.		
434	— 8	95
437	— 10	93
437	— 11	91

Experiment 508.

AB.	BC.	(A + C) B.
volt.	volt.	volt.
0·0450	+ 0·0157	+ 0·0248
497	173	261
512	180	270
525	189	280
535	197	288
550	210	300
560	218	308
CHCl ₃ to BC.		
645	120	230
648	60	100 ?
670	30	180
683	13	167
687	+ 3	159
780	— 3	154
CHCl ₃ off.		
720	— 8	153

The sign + indicates that the current was directed from outer to inner in the skin.

Conclusions.—Chloroform applied to electrode BC—

- (i) Causes no change in the potential between A B (as in Series I).
- (ii) Abolishes the p.d. between B C.
- (iii) Diminishes the p.d. between A + C and B.

For further observations see below.

Series III.

Determinations were made of the electrical resistance of skin before and after chloroformisation, in the same manner as the parallel series in nerve, the skin resting by its external on two non-polarisable electrodes in a chamber to which moist CHCl₃ vapour of about 16 per cent. or moist air could be passed at will. The probable error of a single observation does not exceed 2 per cent.

Conclusions.—Chloroform causes—

- (i) A diminution of real resistance of about 24 per cent.
- (ii) A diminution of polarisation of about 10 per cent.

The first of these conclusions contrasts markedly with the results of the experiments on nerve, where there was no alteration of the real resistance (as opposed to polarisation) within the limits of error of the method.

Skin, CHCl_3 .

Experiment.	Resistance before CHCl_3 .	T°.	Resistance during CHCl_3 .	T°.	Resistance after CHCl_3 .	T°.	R ₂ /R ₁ .	CHCl_3 per cent.
WHEATSTONE.								
145	ohms. 39,300	16·6	ohms. 31,400	...	ohms. 27,500	...	0·700	15
146	28,000	16·9	19,300	...	16,700	...	0·596	15
147	20,340	17·3	13,400	...	14,000	...	0·700	16·5
Mean	29,213	...	21,366	...	19,400	...	0·664	
KOHLEKRAUSCH.								
410 A	21,000	18·7	21,000	19·2	20,800	19·0	0·991	Control thread, M/10 NaCl.
B	18,500		15,100		13,500		0·730	Skin
411 A	19,300	19·0	19,000	19·4	19,000	19·1	0·985	Thread
B	18,700		16,700		14,700		0·768	Skin
412 A	18,300	18·9	18,300	19·5	18,000	19·2	0·984	Thread
B	12,701		11,400		10,200		0·803	Skin
413 A	18,300	19·3	18,300	19·7	18,300	19·3	1·000	Thread (same as 412 A).
B	17,400		13,700		13,400		0·770	Skin
Mean thread	19,230	18·98	19,150	19·45	19,030	19·15	0·990	Mean thread
Mean skin	16,830		14,230		12,950		0·765	Mean skin

Observations and Deductions.

It is to be observed that in these experiments there is no ambiguity (such as is possible in the case of nerve) as to the action of chloroform. Neither an increase or decrease of potential occurs (neglecting for the moment the very small residual effect noted above) when the drug is applied to the electrode B, in contact with the inner surface of the skin; this spot is therefore acting merely as a moist conductor. When the CHCl_3 is applied to the electrode A, which touches the external surface, the current is abolished. It is a necessary consequence that the apparatus giving the current is located at this spot.

This conclusion is in accord with all the classical observations on the resting current (see the authors quoted above); the only other observation that it is necessary to mention here is that of Waller* on the "blaze" current. Using the A B C method, he found that the seat of the electromotive effect was accurately localised at the spot on the external surface touched by the electrode, and that the opposite spot on the internal surface was quite inert. Although one cannot at the present time define exactly the relations existing between these "blaze" currents and the "resting" currents, there is no doubt that there is a close connection between them.

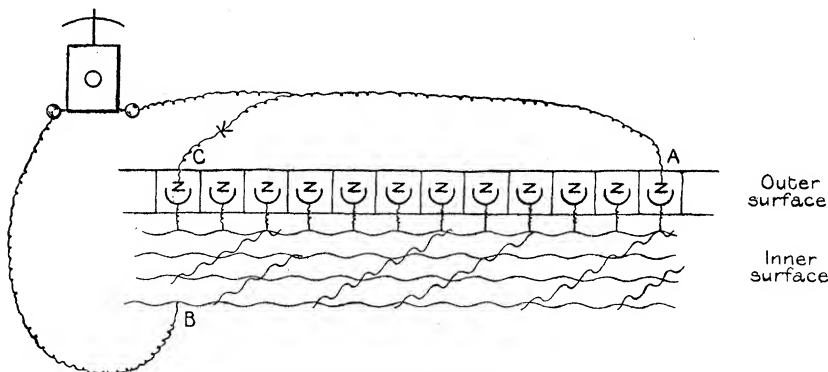


FIG. 4.—Diagram of Electrical Mechanism in the Skin of the Frog.

It is possible to make further deductions from the present experiments. If a diagram (fig. 4) be constructed to reproduce the observed phenomena, it must present the following features:—

- (i) A series of separate cells placed side by side.
- (ii) The positive elements (*e.g.*, zincs) lie towards the outer surface, the negative (*e.g.*, carbons) lie towards the inner.
- (iii) The negative elements are connected with each other.
- (iv) The positive elements are insulated.

* Waller, *loc. cit.*

From the experiments in Series I, the postulate (ii) follows immediately; (i) and (iv) are necessary in this way. CHCl_3 abolishes the potential difference between the outer and inner surfaces, and renders that portion of the tissue isoelectric. If the whole outer surface was in electrical connection, the rendering isoelectric of any one part would cause a short-circuit there, and so diminish the current between any other point A on the outer surface and any point B on the inner surface—the experiments in Series I show that this is not the case. Series II confirms this conclusion. CHCl_3 to BC abolishes the potential difference there; it leaves unaffected AB, and the potential between A—C (in parallel) and B is diminished by an amount dependent on the relative resistance between A, B, and C. For a little distance inwards, therefore, the outer surface of the skin has a very high longitudinal resistance.

The postulate (iii) follows in a similar manner. Further, as the skin as a whole is a good conductor, and as the outer surface is a bad conductor, the inner surface must be a good conductor. As the negative elements are in connection with this tract, they are in connection with each other.

It is not yet possible to say what elements in the skin act in the manner of the (ZnC) cells in the diagram (fig. 4), still less the exact nature of the action. Certain parts of the frog's skin give an alkaline reaction, others an acid, and the relations between the secretion and the resting current (still more the variations on excitation) are yet to be determined.* But if one regards (for the sake of simplicity) only the glands, one can make a tentative picture of the process (fig. 5). The whole gland will resemble one cell in the

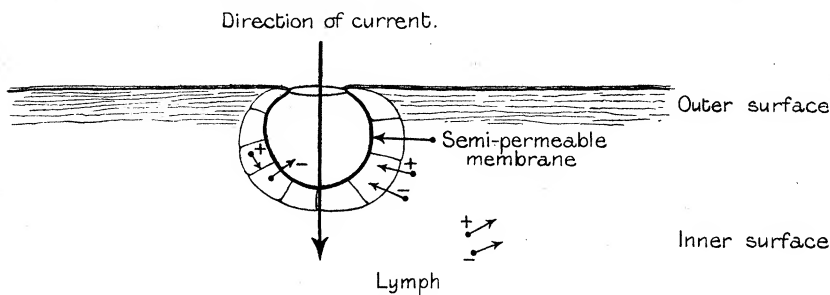


FIG. 5.—Tentative Diagram of Frog's Skin. For simplicity, the membrane (or part of the cell) has been drawn as permeable to $-$ ions. In reality the conditions are more complex.

diagram. As the carbons in fig. 5 are connected together and with the inner surface, so the lymph must bathe the inner surface of the gland and freely enter the cells of the gland. Now if the outer part of these cells—possibly

* See Bayliss and Bradford, *loc. cit.*; also Hermann, 'Pflüger's Archiv,' 1878, vol. 17, p. 291.

the limiting membrane—has a directive or semi-permeable action, letting through some ions more freely than others, the general behaviour of the apparatus would approximate to the scheme. If, then, chloroform destroys the semi-permeable mechanism (perhaps in the manner suggested by Meyer* and Overton†) every sort of ion could wander freely in any direction, and so the whole tissue would become isoelectric. Further, this destruction would be equivalent to a diminution of viscosity, and so would account for the great increase in conductivity observed in Series III.

This must be regarded merely as a sketch of what is possible rather than a finished hypothesis; still this view presents certain problems in a manner that will admit of a solution which will either prove or disprove the suggestions here advanced.

At any rate, the experiments show :—

- (1) That there are certain structures acting as semi-permeable membranes.
- (2) That chloroform inhibits or destroys their functions.

Relations with Part I and Alternative Theories.

It will be remembered that in the consideration of the action of chloroform (and anæsthetics generally) on isolated nerve some phenomena were found which bear a relation to those now under consideration. Summarised they are as follows :—

- (1) CHCl_3 acting on a nerve produces the same electrical effect as an injury.
- (2) CHCl_3 produces less than 2 per cent. alteration in resistance.

If the hypothesis be adopted that there are in the axis cylinder of a nerve certain ions already present, it is plain that the action of the anæsthetic is the same on nerve as on skin, namely, that a semi-permeable apparatus is rendered completely permeable. It is not perhaps clear from the experiments on nerve alone whether this hypothesis is to be adopted or the alternative, that fresh ions are split off from the proteid by the action of the anæsthetic, but as it has been shown above that in the case of skin it is necessary to suppose the existence of a semi-permeable membrane, there is reason to suppose that this is the case in nerve also, and in fact it is not possible to create such a potential difference as is found in the case of nerve without some such arrangement, whether fresh ions are formed or no.‡

* Meyer, 'Schmiedeberg's Archiv,' 1899 and 1901.

† Overton, 'Studien über die Narkose,' Jena, 1901, etc.

‡ On the conditions under which ions can exist in the axis-cylinders of nerve-fibres, see Macdonald, 'Roy. Soc. Proc.,' vol. 76, B, p. 322, and Macallum and Menten, *loc. cit.*, vol. 77, B, p. 165.

It would appear therefore that this action of chloroform, namely, the breaking down of a semi-permeable apparatus, is the characteristic action of an anæsthetic on living tissue.

The additional possibility just mentioned remains to be considered. The view of Moore and Roaf* is that CHCl_3 combines with proteid, forming "unstable compounds or aggregates," and in doing so splits off inorganic ions, further, that "anæsthesia is due to a paralysis of the chemical activities of the protoplasm as a result of the formation of such aggregates." The present experiments (Parts I and II) give no direct information on these subjects, all that can be deduced with certainty is the conclusion given above. Moore and Roaf's theory may be adopted as an addition to this, or it may not. Whether or no this addition is justified by the experimental evidence adduced by these authors is a debatable point.

It is to be remarked that the observations (Part I, Series V and VI, Part II, Series III) on the electrical resistance of the tissues cannot be taken as proving or disproving an ion formation—they show that what is equivalent to a diminution of viscosity takes place (much in skin, little in nerve) and that in the latter case this diminution causes less than 2 per cent. alteration in conductivity, obviously a small production of ions would be masked by these other changes.† As the resistance and freezing-point determinations of Moore and Roaf were made with solutions, there were no membranes to cause this ambiguity, but from inspection of their figures it would appear that the mean differences, which they use in support of their argument, are less than the error of their experiments; it is only a matter of accident what values are obtained in such a case. The question is one that merits further study before a definite conclusion is arrived at.

I have again much pleasure in acknowledging the assistance of Dr. Waller, and I also wish to thank Professor Ayrton for his kindness in examining the electrical data and conclusions. Dr. Collingwood and Mr. Shapiro have again given me much help.

* Moore and Roaf, 'Roy. Soc. Proc.,' vol. 73, p. 382, and vol. 77, B, p. 86.

† Using the apparatus mentioned in the text, it was found that any alteration in the resistance of *serum* was not detectable after CHCl_3 , but that there was a diminution of approximately 15 per cent. in the case of blood (laked or simply defibrinated); it would seem that the effect of CHCl_3 on hæmoglobin would be worth investigating.